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(54) Title: ANTIMICROBIAL TREATMENT OF SAUSAGE CASINGS

(57) Abstract

A shirring solution and its method of use is provided comprising a lower polyalkene glycol characterized by a molecular weight of 600 or greater and a bacteriocin. Also provided is a shirring solution comprising a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity.

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ANTIMICROBIAL TREATMENT OF SAUSAGE CASINGS

This application claims priority on provisional patent application Serial No. 60/113,854 filed December 24, 1998 and provisional patent application Serial No. 60/114,777 filed January 5, 1999.

FIELD OF THE INVENTION

The present invention relates generally to an improved shirring solution for cellulosic sausage casings which when applied to the casings inhibits microbial growth of various pathogens which produce diseases.

BACKGROUND OF THE INVENTION

There are increasingly more serious incidences of foodborne illnesses in the world. These create life-threatening situations. In 1997, there were 143,000 documented foodborne illnesses in the USA, resulting in 600 deaths. The actual number of cases in the USA is estimated at eight million annually. The Center for Disease Control has listed eight main pathogens, or agents responsible for foodborne illness pathogens. These diseases include Campylobacter jejuni, Clostridium bolutlinum, Clostridium perfringens, Escherichia coli 0157, Listeria monocytogenes, Salmonella enteritis, Cagulas positive Staphylocccus aureus, and Yersinia enterocolitica.

Campylobacter jejuni, which causes Campylobacteriosis, is found mainly in raw poultry, raw meat, milk and untreated water. It is generally controlled by cooking above 155° F. Although this is now the most common foodborne illness, it requires hospitalization in only 10% of cases.

Clostridium Bolutinum, which causes botulism, is found mainly in underprocessed canned goods and vacuum packaged food products. It is generally controlled by proper preparation of canned goods.

Clostridium Perfringens, which causes enteritis, is found mainly in improperly cooked meats and poultry. It is generally controlled by cooking above 155° F.

Escherichia Coli 0157:H7 or "E.coli", which causes intestinal illness, is found in raw meat, poultry, fruit, vegetables, impure water and unpasteurized milk. It is generally controlled by cooking above 155° F. E.coli contamination of packaged foods can occur after the packaged food is processed. Therefore, cooking alone is not an insurance against E.coli contamination of food products, especially meats. Hospitalization is usually required in 30% of cases.

Listeria monocytogenes, which causes listeriosis, is found in water, air, soil, raw milk, cheese, meat, poultry and produce. Therefore, it is a very omnipresent pathogen, and in meat processing, Listeria contamination has reached critical proportions. It is generally controlled by cooking above 155° F. But in addition, intense measures must be taken to avoid cross-contamination of exposed food prior to packaging. Listeria is extremely hard to combat since it is found in soil, in animals, in water and air. Any slight contamination can accelerate Listeria. Listeriosis in humans usually requires hospitalization, since it can lead to meningitis. Listeria can function between 37° F. and 113° F, both in aerobic and anaerobic environments. It can survive a pH of 4.2 to 9.6. Since Listeria can be present anywhere in meat plants, including on floors, in wash areas, on the sausage peeler itself, in steam condensates, in the compressed air system, on the walls, and the ceilings, it is perhaps the most difficult pathogen to eradicate. Listeria survives brine chills, water sprays and hoods on steam peelers in frankfurter processing lines, and therefore is a continuing concern for hot dog and sausage manufacturers. Furthermore, Listeria does not alter the appearance or smell of the food.

Salmonella enteritis, which creates salmonellosis, is found in undercooked meat and poultry, raw eggs, unpasteurized milk, salads, chesses. It is generally controlled by cooking above 155° F. Also, immediate refrigeration is required of all susceptible foods.

Cagulase Positive Staphylococcus aureus, or "Staph", which creates Staph infections, is found in high protein cooked foods such as cooked hams, salads, luncheon meats, eggs, etc. It is generally controlled by cooking above 155° F. Also, immediate refrigeration is required of all susceptible foods.

Yersinia enterocolitica, which creates yersiniosis, is found in untreated water, raw meat, seafood, fruits, vegetables, milk and ice cream. It is generally controlled by cooking above 155° F.

It is alarming that 20 years ago, Campylobacter, Listeria, and E.coli were unrecognized as causes of foodborne illness, yet these three, along with salmonella represent the top four causes of foodborne illness. As research intensifies, it has been found that Listeria and yersinia can grow in refrigerated conditions, therefore combating them is not easy. Refrigeration can greatly reduce E.coli populations, but in many cases Listeria will flourish. Listeria will grow at temperatures as low as 3°C, so if food is contaminated prior to packaging, refrigeration does not prevent its growth.

It can be seen, that in many food packaging applications, especially sausage and hot dog processing, E.coli and Listeria contamination is a significant concern. For example, although a product, such as a hot dog, can be processed free of pathogens, contamination of the meat can still occur in many ways prior to the application of protective packaging. Despite increasing measures of protection, there have been numerous well-publicized outbreaks of meat contamination of E. coli and Listeria. Therefore, improvements in meat processing technologies are needed to combat these bacteria. Various technologies currently exist which attempt to treat meats to prevent bacteria formation. However, often in many instances, these technologies are not government approved, or are simply ineffective. For example, antibiotics, such as virginiamycin, spiramycin, tylosin phosphate, and bactiracin-zinc, can be used in animal feeds Nevertheless, the use of antibiotics in animal feeds is banned in many countries. Irradiation of meat products is another possible method for killing food-borne pathogens. However, irradiation is expensive and not widely accepted by the public. Furthermore, the USDA has not yet accepted irradiation for all meat uses.

In the meat processing industry, practices to combat E.coli and Listeria include blasting carcasses with superheated steam and rinsing the carcasses in solutions of hot water and acid. However, the acid rinses often alter the taste characteristics. Furthermore, the rinses are often ineffective against Listeria and salmonella.

Application of liquid smoke to meat has been shown to reduce E. coli. However, this reduction occurs only during the processing stage, and is not present on the meat surface prior to packaging, where meat is vulnerable to E.coli and Listeria contamination.

The application of trisodium phosphate has been effective in reducing bacteria. However, the USDA has approved its use only on carcasses for experimental purposes. Further, some studies have shown that trisodium phosphate is ineffective as a beef sanitizer.

Research has been conducted on hyperpasteurization in which there is first a high vacuum evacuation of food packages, followed by oxygen saturation, followed by a final vacuuming. After a final vacuuming, the inert atmosphere environments are introduced into the package. This is an expensive technology, and requires substantial development efforts.

Technology exists which uses mold inhibitors such as potassium sorbate and propylene glycol to prevent bacteria growth. However, it has been found that these mold inhibitors do not inhibit the growth of pathogenic food borne illnesses such as Listeria and E. coli. Potassium sorbate and propylene glycol will reduce rancidity, mold growth, and yeast formations. In addition, they have been used to effectively reduce water activity in cellulosic sausage casings, therefore removing a water's availability to act as a nutrient for mold growth.

There has been recent interest in the use of antimicrobial additives in packaging films. However, in the case of processed meats, such as hot dogs, packaging is usually in quantities of eight, ten, or twelve hot dogs in a one pound package. The packaging film itself is in intimate contact only with between half and one quarter of the hot dog surface. Hence, it cannot impart antimicrobial properties to the entire surface. Furthermore, additives, such as sorbic acid used in cellophane, or potassium sorbate and parabens used in PVDC films have been ineffective in long term control of Listeria in hot dogs. Organic acids used in films have also been found to be ineffective. The bacteriocin, nicin in packaging films has also been used in packaging films but a large quantity is required. Silver coatings such as Zeolite have

been shown to inhibit bacterial growth in laboratory tests, but have been unsuccessful on the film itself.

Tests have been conducted in which potassium sorbate is blended with low density polyethylene polymer to combat yeasts and improve shelf life. However, the potassium sorbate will not act as a bactericide. Recent research has shown that bifidobacterium bifidum or bifidocin B is effective against Listeria, but has not shown if the bifidocin B is effective when applied to a food packaging material. A new chemical, Sanova (Alcide), has shown to be effective against E. coli and Listeria, but does not yet have USDA approval, and is not yet incorporated in to the packaging film.

Additives are often used in meats to not only preserve and prevent bacterial formation, but also to enhance meat properties, such as taste and color. U.S. Patent No. 4,013,797 discloses the use of lactobacillus or micrococcus to enhance red coloration in meat. U.S. Patent No. 4,147,807 discloses the use of micrococcus varians with pediococcus cerevisae and lactic acid to the lower the pH and improve the red coloration of meat. U.S. Patent No. 4,165,391 discloses the use of whey solids and other chemicals to provide meaty flavors to cheeses. U.S. Patent No. 4,303,868 discloses treating meat with micrococcus varians, pediococcus cerevisae, and lactic acid to improve the meat's flavor. U.S. Patent No. 4,728,518 discloses the use of a streptococcus lactis and nitrate mixture to create a red meat color. U.S. Patent No. 4,847,097 discloses the use of streptococcus lactic to create a rapid red meat coloration.

Additionally, additives are often used to preserve or prevent bacterial growth in meats. U.S. Patent No. 3,794,739 discloses lactic acid in foods to inhibit food poisoning bacteria. U.S. Patent No. 3,960,664 discloses the use of pediococcus cerevisae and glycerol in fermented meats. U.S. Patent No. 4,238,513 discloses the use of pediococcus pentosaceus to control meat pH in semi dry sausage for fermentation control. U.S. Patent No. 4,303,679 discloses the use of lactic acid cultures of pediococcus penosaceus for low temperature meat preservation, to reduce the use of nitrate curing compounds in dry sausage. U.S. Patent No. 4,407,828 discloses the use of lactobacillus acid development for low temperature fermentation. U.S. Patent No. 4,477,471 discloses the use of streptococcus lactis for food

preservation. U.S. Patent No. 4,492,712 discloses the use of a hydrolyzed whey extender in sausage to reduce water activity and pH. U.S. Patent No. 4,514,424 discloses the use of lactobacillus casei for low temperature fermentation. U.S. Patent No. 4,810,508 discloses adding lysozyme to foods to reduce Listeria. U.S. Patent No. 4,867,990 discloses the use of lactate monooxygenase to prevent oxygen deterioration. U.S. Patent No. 4,874,704 discloses inoculating lactobacillus into food to reduce Listeria and salmonella. U.S. Patent No. 4,929,445 discloses inoculating pediococcus acidilacti into food to inhibit Listeria. U.S. Patent No. 4,980,163 discloses the use of lysostaphin, lanthione, and peptide bacteriosin for bacterial reduction. U.S. Patent No. 5,085,873 discloses the use of a surface coating of lactoperoxidase, thiocynate, and an oxygen donor to reduce Listeria. U.S. Patent No. 5,217,950 discloses the use of lanthionin bacteriosins. U.S. Patent No. 5,268,185 discloses treating meat with trialkali metal orthophosphate to reduce Listeria and other pathogens present in the meat without creating a color change. U.S. Patent No. 5,348,881 discloses the use of lactococcus bacteriocins in food. U.S. Patent No. 5,455,038 discloses adding tetrahydroisohumulone to food to reduce Listeria.

Tubular food casings provide an excellent method of providing an antimicrobial bactericide to the processed meats. A processed meat is sterilized during cooking. The exposure to E. coli and Listeria is most severe when the casing is removed prior to packaging. Therefore, a surface treatment of an effective bactericide approved for food use would greatly eliminate any surface contamination of the meat, providing an effective coating on the surface prior to packaging.

Of particular interest are Wilhoit, U.S. Patent Nos. 5,573,797, 5,573,800, and 5,573,800 which disclose the use of an antimicrobial composition such as a streptococcus or pediococcus bacteriocin in combination with a chelating agent to protect foodstuffs against the growth of harmful bacteria such as Listeria. The patents further disclose the use of the antimicrobial compositions on food packaging films, such as cellulosic food casings, although the presence of the chelating agent was required to achieve satisfactory results. The use of chelating agents, such as those comprising heavy metals is often undesirable in foodstuffs. It would therefore, be

desirable to provide a shirring solution that contains a bactericide that satisfactorily inhibits bacterial growth, but which is also free of any chelating agents.

SUMMARY OF THE INVENTION

The invention provides an improved method of shirring cellulosic sausage casings wherein the shirring solution contains a bacteriocin and a lower polyalkene glycol characterized by a molecular weight of at least 600 which prevents bacterial growth on the meat when it is applied to the interior surface of the casing. The polyalkene glycol is preferably selected from the group consisting of polyethylene glycol and polypropylene glycol and is preferably a polyethylene glycol characterized by a molecular weight of from 800 to 1100 with a molecular weight of about 900 being particularly preferred. The polyalkene glycol preferably comprises 5% and, more preferably 10% by weight of the shirring solution. Preferred bacteriocins for use according to the invention are preferably those derived from members of the group consisting of lactobacillus, pediococcus, leuconostoc, and streptococcus and their synthetic equivalents and are preferably applied at concentrations of from about 0.5% to about 5% with concentrations of from about 1% to 3% being particularly preferred.

The invention further provides shirring solutions comprising a bacteriocin and a lower polyalkene glycol characterized by a molecular weight of at least 600 and casings produced by shirring with the solution. The invention also provides improved sausage products characterized by increased resistance to Listeria infection produced by the methods of the invention.

According to a further embodiment of the invention an improved method of shirring cellulosic sausage casings is provided wherein the shirring solution comprises the combination of a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin which is a different bacteriocin from the first having anti-gram-negative bactericidal activity. According to one aspect of the invention the first bacteriocin is derived from Pediococcus. One preferred such bacteriocin is Alta® 2341 available from Quest International, Hoffman Estates, IL. which is particularly effective against gram-positive bacteria such as Listeria and Streptococcus. According to another aspect of the invention, the first bacteriocin is derived from Lactococcus.

One preferred such bacteriocin is Perlac® 1911 available from Quest International, Hoffman Estates, IL. which is particularly effective against gram-negative bacteria. When used in combination, the anti-gram-negative and anti-gram-positive bacteriocins are particularly effective in preventing Listeriosis in treated sausage. As will be recognized by those of skill in the art, many bacteriocins are characterized by antibacterial activity against both gram-positive and gram-negative organisms. Therefore, as used herein the recitation of a first bacteriocin having anti-gram-positive bactericidal activity encompasses those bacteriocins which also have anti-gram-negative bactericidal activities. Similarly, the recitation of a second bacteriocin having anti-gram-negative bactericidal activity includes those bacteriocins which also have anti-gram-positive bactericidal activity although it is preferred that the anti-gram-negative bactericidal activity be substantially effective at killing gram-negative bacteria and also that it exceed the anti-gram-positive activity of the second bacteriocin. A particularly preferred combination is that of the Alta® 2341 bacteriocin with the Perlac® 1911 bacteriocin. Another preferred combination of anti-gram-negative and anti-gram-positive bacteriocins is available commercially as Altamate® from Quest International, Hoffman Estates, IL. which comprises the Alta® 2341 bacteriocin and the Perlac® 1911 bacteriocin in further combination with propionic acid.

The invention further provides shirring solutions comprising a combination of a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity. The invention also provides improved sausage products characterized by increased resistance to Listeria infection produced by the methods of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides the use of bacteriocins and a lower polyalkene glycol as shirring solution additives for cellulose casings which penetrate the meat surface and prevent bacteria formation. Bacteriocins are protein substances released by certain bacteria that kill, but do not lyse closely related strains of bacteria.

Specifically, preferred bacteriosins used are derived from members of the group consisting of lactobacillus, pediococcus, leuconostoc, streptococcus, and their

synthetic equivalents. Preferred bacteriocins for use according to the invention are commercially available as Alta 2341 and Per/Lac 1901. (Quest International) Preferred lower polyalkene glycols for use according to the invention include C_2 to C_5 polyalkene glycols with polyethylene glycol and polypropylene glycol being particularly preferred. Preferred polyethylene glycols for use according to the invention are those having molecular weights of about 600 or about 900 which are commercially available as Carbowax® 600 or Carbowax® 900, respectively (Union Carbide).

As one aspect of the invention, it has been found that antibacterial agents such as bacteriocins when applied to a sausage casing in a shirring solution according to prior art method have the tendency to migrate into the cellulosic casing and away from the meat of the sausage. Because, the casing is eventually peeled from the sausage a substantial portion of the anti-Listeria protective capacity of the bacteriocin is thereby lost. As another aspect of the invention, it has been found that when a relatively high molecular weight lower polyalkene glycol component is incorporated into the shirring solution, the bacteriocin component of the shirring solution is more effectively transferred to the meat of the sausage than in the absence of such a component.

The present invention provides the use of combinations of a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity. As one aspect of the invention it is contemplated that combinations of anti-gram-positive and anti-gram-negative bacteriocins in shirring solutions will be synergistically effective in the prevention of Listeriosis compared to equivalent shirring solutions comprising only anti-gram-positive bacteriocins.

In addition to the lubricants and other ingredients which conventionally make up shirring solutions, the shirring solutions of the invention may comprise additional ingredients which promote the antibacterial effects of the solutions. Such ingredients include lower polyalkene glycols which promote the transfer of the bacteriocins to the meat prior to pealing of the sausage casing. Preferred lower polyalkene glycols for use according to the invention include C_2 to C_5 polyalkene glycols with polyethylene glycol and polypropylene glycol being particularly preferred.

Preferred polyethylene glycols for use according to the invention are those having molecular weights of about 600 or about 900 which are commercially available as Carbowax® 600 or Carbowax® 900, respectively (Union Carbide).

According to this aspect of the invention, it has been found that antibacterial agents such as bacteriocins when applied to a sausage casing in a shirring solution according to prior art method have the tendency to migrate into the cellulosic casing and away from the meat of the sausage. Because, the casing is eventually peeled from the sausage a substantial portion of the anti-Listeria protective capacity of the bacteriocins is thereby lost. As another aspect of the invention, it has been found that when a relatively high molecular weight lower polyalkene glycol component is incorporated into the shirring solution, the bacteriocin components of the shirring solution are more effectively transferred to the meat of the sausage than in the absence of such a component.

Without intending to be bound by a particular theory of the invention, it is believed that the lower polyalkene glycol binds with water present in the cellulosic casing, which in effect creates a layer of the lower polyalkene glycol that is in contact with the casing, followed by a second layer of carboxymethylcellulose (when present in the shirring solution), and finally a third layer of bacteriocin which is in direct contact with the meat. As a result, the shirring solution maintains its bacteriocin component in direct contact with the meat, avoiding the need for chelating agents to "bind" with the meat and bacteriocin. Thus, when the casing is peeled from the sausage, a substantial portion of the bacteriocin remains in contact with the meat.

In addition to the use of lower polyalkene glycols as additional shirring solution ingredients the shirring solutions may alternatively or in addition comprise chelating agents such as ethylene diamine tetraacetic acid (EDTA) such as disclosed in Wilhoit, U.S. Patent Nos. 5,573,797, 5,573,800, and 5,573,801 the disclosures of which are hereby incorporated by reference.

EXAMPLE 1

According to this example, five different shirring solutions were prepared and applied to regenerated cellulose sausage casings (Alfacel), size 27 x 110,

with a casing flatwidth of 36.2 mm. The control casing composition was 73.6% by weight cellulose, 12.2% by weight glycerine, and 11.8% by weight water. The cellulose casing was shirred using conventional shirring techniques to compress a 110 foot stick to 15 inches long.

The control solution was a conventional shirring solution with no bacteriocins present. Shirring solutions were then prepared with either Alta 2341 bacteriocin or Perlac 1901 bacteriocin solutions being added at both 1% by weight and 3% by weight according to Table 1 below. The shirring solutions mixed easily and were then applied to the casing inner surfaces at deposition rates of 30.6 mg per 100 cm^2 with internal mineral oil coverage of 3.24 mg per cm^2 and external mineral oil coverage of 9.30 mg per 100 cm^2. The casings were then allowed to age 48 hours to equilibrate. Two tests were then conducted with these treated casings. See Table I below for compositions of the solutions.

TABLE I

Shirring Solution/ Ingredients	1 Control %	2 1% Alta %	3 1% Perlac %	4 3% Alta %	5 3% Perlac %
Water	85	85	85	85	85
Polysorbate 80 (Tween® 80)	0.26	0.26	0.26	0.26	0.26
Sorbitan Oleate (Span® 80)	0.15	0.15	0.15	0.15	0.15
Carboxymethyl cellulose (CMC-7LF®)	2.00	2.00	2.00	2.00	2.00
Methylcellulose (Methocel®)	0.25	0.25	0.25	0.25	0.25
Propylene Glycol	0	0	0	0	0
Bacteriocin (Alta 2341)	0	1.00	0	3.00	0
Bacteriocin (Perlac 1901)	0	0	1.00	0	3.00
Lecithin	0.25	0.25	0.25	0.25	0.25
Polyethylene glycol (Carbowax® 600)	12.09	11.09	11.09	9.09	9.09

Shirring solutions 1-3 were each applied to the casings described above. After 48 hours, the casings were seeded with E. coli, Staphylococcus, Listeria, and Salmonella. Two methods were used to seed the samples with bacteria. In the first method, bacteria were grown in enriched broth (tryptic soy broth (TSB)). The bacteria were harvested by centrifugation and washed with 0.9% saline. The bacteria were diluted in TSB prepared at 10% of the normal strength so that the absorbance of the bacterial suspension at 600 nm was 0.1-0.2. Ten milliliters of each suspension was placed in a 10 cm length of casing and loosely closed. Samples were incubated at room

temperature. The casings were incubated so that as much of the broth was in contact with the casing wall as possible. Aliquots of the broth were removed at 12, 24, and 48 hours and the number of bacteria determined.

In the second method of inoculating the casing with bacteria, the bacterial suspensions were prepared as in method one and were spread on the interior of a sample of opened casing with a sterile sponge. The resulting samples were placed in a sterile container and allowed to incubate at room temperature for 24 hours. Following the 24 hours of incubation, the samples of inoculated casing were placed in 99 ml sterile buffer and shaken. One ml aliquots of the buffer were used to inoculate Plate Count Agar for enumeration of the bacteria present.

Following the seeding with bacteria, all samples were incubated under conditions selected to promote bacteria growth. In 48 hours, the bacterial counts on the control casing (solution 1) generally stabilized or grew. However shirring solution 2, which contained the Alta 2341 bacteriocin showed a considerable reduction in 48 hours of the E.coli, Staph, and Listeria, as well as a smaller reduction in the salmonella. Shirring solution 3, which contained the Perlac 1901 bacteriocin also showed some inhibitory quality. The results of the tests are shown in Tables II-V below.

TABLE II- METHOD 1

CONTROL

Time (hrs)	Bacterial Count (cfu/ml)			
Bacteria	*E. coli.*	*S. aureus*	*L. Monocytogenes*	*S. enteriditis*
0	5.6×10^3	6.6×10^3	4.7×10^3	4.8×10^3
12	7.2×10^3	8.1×10^3	6.2×10^3	6.8×10^3
24	8.9×10^4	9.3×10^4	4.1×10^4	3.4×10^4
48	7.0×10^3	5.7×10^3	7.9×10^3	9.9×10^3

TABLE III- METHOD 1

1% ALTA BACTERIOCIN

Time (hrs)	Bacterial Count (cfu/ml)			
Bacteria	*E. coli.*	*S. aureus*	*L. Monocytogenes*	*S. enteriditis*
0	5.6×10^3	6.6×10^3	4.7×10^3	4.8×10^3
12	5.8×10^3	7.1×10^3	5.8×10^3	5.4×10^3
24	8.1×10^4	1.1×10^4	1.9×10^4	2.1×10^4
48	1.4×10^3	2.8×10^3	3.4×10^3	3.7×10^3

TABLE IV-METHOD 1

1% PERLAC BACTERIOCIN

Time (hrs)	Bacterial Count (cfu/ml)			
Bacteria	*E. coli.*	*S. aureus*	*L. Monocytogenes*	*S. enteriditis*
0	5.8×10^3	6.4×10^3	4.9×10^3	4.6×10^3
12	5.9×10^3	6.1×10^3	5.4×10^3	5.9×10^3
24	9.4×10^4	1.7×10^4	1.2×10^4	1.7×10^4
48	3.8×10^3	5.8×10^3	6.7×10^3	4.9×10^3

TABLE V- METHOD 2

Organism	Control		1% Alta Bacteriocin		1% Perlac Bacteriocin	
Time (hrs)	0	24	0	24	0	24
E. coli.	1250	1100	1380	960	1360	1110
S. aureus	1180	1050	1240	870	1180	910
L. Monocytogenes	2120	2010	2150	1750	2160	1800
S. enteriditis	1540	1470	1610	1480	1550	1510

EXAMPLE 2

According to this example, shirring solutions 1, 4 and 5 prepared as in Example 1 were each applied to the casings also used and described in Example 1. After 48 hours, the casings were seeded with E. coli, Staphylococcus, Listeria, and

Salmonella according to the two methods described above. Again, the Alta 2341 bacteriocin showed a considerable reduction in 48 hours of E. coli. Staph, and Listeria, and a smaller reduction in the salmonella. The Perlac bacteriocin also showed some inhibitory quality, but it was not as good as the Alta bacteriocin. The results are shown below in Tables VI-VIII.

TABLE VI-METHOD 1

3% ALTA BACTERIOCIN

Time (hrs)	Bacterial Count (cfu/ml)			
Bacteria	*E. coli.*	*S. aureus*	*L. Monocytogenes*	*S. enteriditis*
0	5.6×10^3	6.6×10^3	4.7×10^3	4.8×10^3
12	5.6×10^3	6.4×10^3	4.8×10^3	5.5×10^3
24	7.5×10^4	8.9×10^3	1.4×10^4	1.1×10^4
48	1.0×10^3	2.4×10^3	2.9×10^3	3.5×10^3

TABLE VII- METHOD 1

3% PERLAC BACTERIOCIN

Time (hrs)	Bacterial Count (cfu/ml)			
Bacteria	*E. coli.*	*S. aureus*	*L. Monocytogenes*	*S. enteriditis*
0	5.6×10^3	6.6×10^3	4.7×10^3	4.8×10^3
12	5.4×10^3	6.2×10^3	4.1×10^3	4.9×10^3
24	6.8×10^4	9.1×10^3	1.9×10^4	1.4×10^4
48	3.8×10^3	4.7×10^3	4.1×10^3	5.4×10^3

TABLE VIII- METHOD 2

Organism	Control		3% Alta Bacteriocin		3% Perlac Bacteriocin	
Time	0	24	0	24	0	24
E. coli	1250	1100	1320	780	1340	810
S. auereus	1180	1050	1190	790	1200	830
L. monocytogenes	2120	2010	2090	1430	2110	1310
S. enteriditis	1540	1470	1570	1320	1510	1380

EXAMPLE 3

According to this example, a third test was conducted in which shirring solutions 1, 4 and 5 were applied to the casings. The shirring spray was delivered at a rate of 76.5 mg/100 cm^2. In total, approximately 2.3 mg of bacteriocin was transmitted to a sausage at a maximum size of 19.5 mm in diameter by 5.5 inches long. The three casings were then shirred and sent to be processed. A theoretical calculation shows that when a shirring solution that has 3% Alta or Perlac bacteriocin, if 100% of the chemical is transferred from the casing to the surface of a hot dog, the loading on the hot dog surface would be about 0.17% Alta or Perlac in a surface layer with depth penetration of 0.05 mm into the surface. The actual penetration of the bacteriocin was unknown, but it is anticipated to be no more than 0.05 mm, depending upon varying porosity and water content of the meat.

The casings were filled to 19.5 mm diameter, using a 9.75 mm outer diameter horn and a #19 chuck with a standard all-chicken meat emulsion on a Townsend RT7 sausage stuffer. The emulsion was at 62° F. The smokehouse temperatures were 140° F for 5 minutes with no humidity, then 160° F Dry Bulb ("DB")/130°F Wet Bulb ("WB") for 15 minutes at 42% relative humidity, then 193° F DB/148°WB for 20 minutes at 32% relative humidity, followed by a hot water shower for 5 minutes, and a chill with brine for 15 minutes. During smoking, the product had liquid smoke added in the meat emulsion.

The final cooked hot dog had a link of 34 g, containing 6 g of fat, 20 mg of cholesterol, 370 mg of sodium, 1 g of carbohydrate, 0 g fiber, 0 g sugar, and 6 g protein.

The hot dogs were handled in two methods. In the first method, the casings were peeled and the meat samples containing the Alta 2341 and Perlac 1901 bacteriocins treated product were packaged in vacuum packs. In the second method, the meat and casing were left together. Non-treated meat samples were also collected in vacuum packs. All of these samples were analyzed for Listeria and E.coli and found to be negative.

The packages were then opened and the frankfurter surfaces and interiors were inoculated with E.coli and Listeria by two different methods. In the first method, bacteria was grown in an enriched broth (Tryptic Soy Broth (TSB)). The bacteria were harvested by centrifugation and washed with 0.9% saline. The bacteria were diluted in TSB prepared at 10% of the normal strength so that the absorbance of the bacterial suspension at 600 nm was 0.1-0.2. One frankfurter of each treatment type (control, Alta, and Perlac) were laid side by side on a sterile surface. The bacteria were applied to the surface of the franks with a sterile brush so that all received the same coverage. The samples were incubated overnight at room temperature. After incubation, a section of each frank was removed, diluted 1:10 in a sterile buffer, homogenized and plated on LPM agar for Listeria enumeration and coliform/E. coli petrifilm (3M) for enumeration of E. coli.

In the second method, the bacterial suspensions were prepared as above. 0.1 ml of each bacterial suspension was injected into the interior of each sample with a hypodermic syringe. These samples were placed in a sterile container and were allowed to incubate at room temperature overnight. The samples were then examined in the same manner as in Method 1.

The amount of the Alta 2341 bacteriocin or Perlac 1901 bacteriocin which transferred to the surface was unknown. It was found the Listeria and E.coli count in the interior of the meat grew substantially, as expected. However, approximately 12 hours after inoculation, the surface of the meat displayed a dramatic reduction in E.coli and Listeria, both with Alta 2341 and Perlac 1901. In twelve

hours, the Alta 2341 reduced the amount of E.coli present on the surface of the meat by 80%, and reduced Listeria by 75%. The Perlac 1901 reduced E.coli by 8%, and reduced Listeria by 22%. The hot dogs made with the shirring solutions containing the Alta bacteriocin and Perlac bacteriocin showed no color change or taste difference. In addition, both casings peeled well. Tables IX and X show the results of test 3 using both test methods 1 and 2.

TABLE IX- METHOD 1

Treatment	Bacterial count (cfu/gm)	
	E. coli	*L. monocytogenes*
Control	5.1×10^4	1250
Alta	9.7×10^3	320
Perlac	4.7×10^4	980

TABLE X- METHOD 2

Treatment	Bacterial count (cfu/gm)	
	E. coli	*L. monocytogenes*
Control	6.1×10^4	7.8×10^3
Alta	5.8×10^4	8.1×10^3
Perlac	5.9×10^4	7.4×10^4

EXAMPLE 4

According to this example, a shirring solutions is prepared and applied to regenerated cellulose sausage casings (Alfacel), size 27 x 110, with a casing flatwidth of 36.2 mm. The control casing composition is 73.6% by weight cellulose, 12.2% by weight glycerine, and 11.8% by weight water. The cellulose casing is shirred using conventional shirring techniques to compress a 110 foot stick to 15 inches long.

Shirring solutions are prepared comprising 85 parts water, 0.26 parts polysorbate 80 (Tween® 80), 0.15 parts Sorbitan Oleate (Span® 80), 2 parts

Carboxymethylcellulose (CMC-7LF®), 0.25 parts Methylcellulose (Methocel®), 0.25 parts lecithin, and 12 parts Polyethylene glycol (Carbowax® 600) are then prepared with either Alta® 2341 bacteriocin or Perlac® 1901 bacteriocin solutions being added alone at both 1% by weight and 3% by weight or in combination together at 0.5% by weight each or 1.5% by weight each. The shirring solutions are mixed, then applied to the casing inner surfaces at deposition rates of 30.6 mg per 100 cm^2 with internal mineral oil coverage of 3.24 mg per cm^2 and external mineral oil coverage of 9.30 mg per 100 cm^2. The casings are then allowed to age 48 hours to equilibrate. Two tests are then conducted with these treated casings.

Shirring solutions are applied to the casings described above. After 48 hours, the casings are seeded with E. coli, Staphylococcus, Listeria, and Salmonella. Two methods are used to seed the samples with bacteria. In the first method, bacteria are grown in enriched broth (tryptic soy broth (TSB)). The bacteria are harvested by centrifugation and washed with 0.9% saline. The bacteria are diluted in TSB prepared at 10% of the normal strength so that the absorbance of the bacterial suspension at 600 nm was 0.1-0.2. Ten milliliters of each suspension are placed in a 10 cm length of casing and loosely closed. Samples are incubated at room temperature. The casings are incubated so that as much of the broth was in contact with the casing wall as possible. Aliquots of the broth are removed at 12, 24, and 48 hours and the number of bacteria determined.

In the second method of inoculating the casing with bacteria, the bacterial suspensions are prepared as in method one and are spread on the interior of a sample of opened casing with a sterile sponge. The resulting samples are placed in a sterile container and allowed to incubate at room temperature for 24 hours. Following the 24 hours of incubation, the samples of inoculated casing are placed in 99 ml sterile buffer and shaken. One ml aliquots of the buffer are used to inoculate Plate Count Agar for enumeration of the bacteria present. Following the seeding with bacteria, all samples are incubated under conditions selected to promote bacteria growth and are evaluated at various time points.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the presently

preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

WHAT IS CLAIMED IS:

1. A method of shirring cellulosic sausage casings comprising applying a shirring solution to the casing, wherein the solution comprises a bacteriocin and a lower polyalkene glycol characterized by a molecular weight of at least 600.

2. The method of claim 1 wherein the lower polyalkene glycol is selected from the group consisting of polyethylene glycol and polypropylene glycol.

3. The method of claim 1 wherein the lower polyalkene glycol is polyethylene glycol characterized by a molecular weight of from 800 to 1100.

4. The method of claim 1 wherein the shirring solution comprises from about 0.5% to about 5% bacteriocin.

5. The method of claim 1 wherein the shirring solution comprises from about 1% to about 3% bacteriocin.

6. The method of claim 1 wherein the bacteriocin is derived from members of the group consisting of lactobacillus, pediococcus, leuconostoc, and streptococcus.

7. A method of shirring cellulosic sausage casings comprising applying a shirring solution to the casing, wherein the solution comprises about 0.5% to about 5% by weight bacteriocin and polyethylene glycol.

8. The method of claim 1 wherein the solution comprises a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity.

9. The method of claim 8 wherein the first bacteriocin is derived from Pediococcus.

10. The method of claim 8 wherein the second bacteriocin is derived from Lactococcus.

11. A shirring solution for cellulosic sausage casing comprising a bacteriocin and a lower polyalkene glycol characterized by a molecular weight of at least 600.

12. The shirring solution of claim 11 wherein the lower polyalkene glycol is selected from the group consisting of polyethylene glycol and polypropylene glycol.

13. The shirring solution of claim 11 wherein the lower polyalkene glycol is polyethylene glycol characterized by a molecular weight of from 800 to 1100.

14. The shirring solution of claim 11 wherein the shirring solution comprises from about 0.5% to about 5% bacteriocin.

15. The shirring solution of claim 11 wherein the shirring solution comprises from about 1% to about 3% bacteriocin.

16. The shirring solution of claim 11 wherein the bacteriocin is derived from members of the group consisting of lactobacillus, pediococcus, leuconostoc, and streptococcus.

17. The shirring solution of claim 11 wherein the solution comprises a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity.

18. The shirring solution of claim 17 wherein the first bacteriocin is derived from Pediococcus.

19. The shirring solution of claim 17 wherein the second bacteriocin is derived from Lactococcus.

20. A sausage produced according to the method of shirring sausage casings comprising applying a shirring solution to the casing, wherein the solution comprises a bacteriocin and a lower polyalkene glycol characterized by a molecular weight of at least 600, and removing the casing from the sausage by peeling.

21. The sausage of claim 20 wherein the lower polyalkene glycol is selected from the group consisting of polyethylene glycol and polypropylene glycol.

22. The sausage of claim 20 wherein the lower polyalkene glycol is polyethylene glycol characterized by a molecular weight of from 800 to 1100.

23. The sausage of claim 20 wherein the shirring solution comprises from about 0.5% to about 5% bacteriocin.

24. The sausage of claim 20 wherein the shirring solution comprises from about 1% to about 3% bacteriocin.

25. The sausage of claim 20 wherein the bacteriocin is derived from members of the group consisting of lactobacillus, pediococcus, leuconostoc, and streptococcus.

26. The sausage of claim 20 wherein the shirring solution comprises a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity.

27. The sausage of claim 26 wherein the first bacteriocin is derived from Pediococcus.

28. The sausage of claim 26 wherein the second bacteriocin is derived from Lactococcus.

29. A cellulosic sausage casing shirred with a shirring solution comprising a bacteriocin and a lower polyalkene glycol characterized by a molecular weight of at least 600.

30. The cellulosic sausage casing of claim 29 wherein the lower polyalkene glycol is selected from the group consisting of polyethylene glycol and polypropylene glycol.

31. The cellulosic sausage casing of claim 29 wherein the lower polyalkene glycol is polyethylene glycol characterized by a molecular weight of from 800 to 1100.

32. The cellulosic sausage casing of claim 29 wherein the shirring solution comprises from about 0.5% to about 5% bacteriocin.

33. The cellulosic sausage casing of claim 29 wherein the shirring solution comprises from about 1% to about 3% bacteriocin.

34. The cellulosic sausage casing of claim 29 wherein the bacteriocin is derived from members of the group consisting of lactobacillus, pediococcus, leuconostoc, and streptococcus.

35. The cellulosic sausage casing of claim 29 wherein the shirring solution comprises a first bacteriocin having anti-gram-positive bactericidal activity and a second bacteriocin having anti-gram-negative bactericidal activity.

36. The sausage of claim 35 wherein the first bacteriocin is derived from Pediococcus.

37. The sausage of claim 35 wherein the second bacteriocin is derived from Lactococcus.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30637

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A23L 3/3463
US CL :426/106, 61, 133, 310, 321, 323, 324, 532, 42
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/106, 61, 133, 310, 321, 323, 324, 532, 42

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US 5,573,797 A (WILHOIT) 12 November 1996, col. 3, lines 35-50.	1, 2, 6, 8, 9, 20, 21, 25-27, 29-31, 34-36
Y,P	US 5,817,357 A (VANDENBERGH et al) 06 October 1998, col. 2, lines 1-10.	3-5, 7, 10, 11-19, 22-24, 28, 32, 33, 37
Y	US 3,898,348 A (CHIU et al) 05 August 1975, col. 4, lines 1-20.	1-37
Y	US 3,124,468 A (WILLIAMS et al) 10 March 1964, col. 2, lines 5-25.	1-7
Y	US 4,867,204 A (ELLIS et al) 19 September 1989, col. 2, lines 10-20.	1, 7, 13, 20, 29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search	Date of mailing of the international search report
08 MARCH 2000	31 MAR 2000

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